Modeling mechanisms of cell secretion

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Abstract

Secretion is a fundamental cellular process involving the regulated release of intracellular products from cells. Physiological functions such as neurotransmission, or the release of hormones and digestive enzymes, are all governed by cell secretion. Anomalies in the processes involved in secretion contribute to the development and progression of diseases such as diabetes and other hormonal disorders. To unravel the mechanisms that govern such diseases, it is essential to understand how hormones, growth factors and neurotransmitters are synthesised and processed, and how their signals are recognized, amplified and transmitted by intracellular signaling pathways in the target cells. Here, we discuss diverse aspects of the detailed mechanisms involved in secretion in light of mathematical models. The models range from stochastic ones describing the trafficking of secretory vesicles to deterministic ones investigating the regulation of cellular processes that underlie hormonal secretion. In all cases, the models are closely related to experimental results and suggest theoretical predictions for the secretion mechanisms.

Key words:Mathematical Model, Vesicles, Bifurcation Analysis, Exocytosis, Hormone Secretion

1 Introduction

An important goal in cell biology is to understand how physiological function is translated into signaling or secretion processes and vice-versa. We have selected different aspects of the mechanisms involved in secretion and focus on the development of mathematical models that can inform experiments and help to understand the details of the underlying processes. Hormone secretion is a complex process, which involves the packaging of the hormone in secretory vesicles, transportation to the cell membrane and calcium-triggered exocytosis. We present mathematical models of hormone secretion from a range of cell types and focus on various steps of the secretion pathway. The next section presents kinetic compartmental models of the insulin secretory granules in pancreatic β-cells; it also serves as a framework for the following sections. Section 3 introduces a stochastic model of the transportation of single secretory granules. Finally, the regulation of the calcium concentration (the main signal for exocytosis and hormone release) in two types of pituitary cells is studied in Section 4. We end with a short conclusion in Section 5.

2 Modeling of insulin secretion

Pancreatic β-cells are responsible for secretion of insulin in response to elevated plasma glucose levels, and the malfunctioning of these cells is a major contributor to the development of diabetes. The β-cells show bursting electrical activity and oscillatory calcium levels and insulin secretion. Mathematical modeling has contributed significantly to the understanding of the generation of these rhythmic patterns; for reviews, see [5, 23]. However, surprisingly little work has been done on modeling insulin secretion, which is the actual task of the β-cell. Already in the 1970's, Grodsky [15] and Cerasi *et al.* [10], among others, did model the pancreatic insulin response to various kinds of glucose stimuli, but these models were phenomenological due to the limited knowledge of the β-cell biology at that time. Only recently has our knowledge of the control of the movement and fusion of insulin granules increased to a level where we have started to formulate mechanistically-based models.

Grodsky [15] proposed that insulin was located in "packets", plausibly the insulin containing granules, but also possibly entire β-cells. Some of the insulin was stored in a reserve pool, while other insulin packets were located in a labile pool, ready for release in response to glucose. The la-

bile pool is responsible for the first phase of insulin secretion [15], while the reserve pool is responsible for creating a sustained second phase. This basic distinction has at least been partly confirmed when the packets are identified with granules [12, 22]. Moreover, Grodsky [15] assumed that the labile pool is heterogeneous in the sense that the packets in the pool have different thresholds with respect to glucose, beyond which they release their content. This assumption was necessary for explaining the so-called staircase experiment, where the glucose concentration was stepped up and each step gave rise to a peak of insulin. There is no supporting evidence for granules having different thresholds [21], but Grodsky [15] mentioned that cells apparently have different thresholds based on electro-physiological measurements. Later, Jonkers and Henquin [18] showed that the number of active cells is a sigmoidal function of the glucose concentration, as assumed by Grodsky [15] for the threshold distribution.

Recently, we showed how to unify the threshold distribution for cells with the pool description for granules [24], thus providing an updated version of Grodsky's model, which takes into account more of the recent knowledge of β-cell biology. An overview of the model is given in Fig. 1. It includes mobilization of secretory granules from a very large reserve pool to the cell periphery, where they attach to the plasma membrane (docking). The granules can mature further (priming) and attach to calcium channels, thus entering the 'readily releasable pool' (RRP). Calcium influx provides the signal triggering membrane fusion, and the insulin molecules can then be released to the extracellular space. We also included the possibility of so-called kiss-and-run exocytosis, where the fusion pore reseals before the granule cargo is released. For the mathematical formulation we united the mobilized and docked pools in a single 'intermediate pool' (Fig. 1b). The glucose-dependent increase in the number of cells showing a calcium signal [18] was included by distinguishing between readily releasable granules in silent and active cells. Therefore, the RRP is heterogeneous in the sense that only granules residing in cells with a threshold for calcium activity below the ambient glucose concentration are allowed to fuse. Hence, our model provides a biologically-founded explanation for the heterogeneity assumed by Grodsky [15] and it is able to simulate the characteristic biphasic insulin secretion pattern in response to a step in glucose stimulation, as well as the secretory profile of the staircase stimulation protocol.

Another property of the pancreas that was implicitly included in Grodsky's model is so-called derivative control, i.e., the fact that the pancreas senses not only the glucose concentration but also the rate-of-change. Modeling of the whole body system has shown that this property is necessary

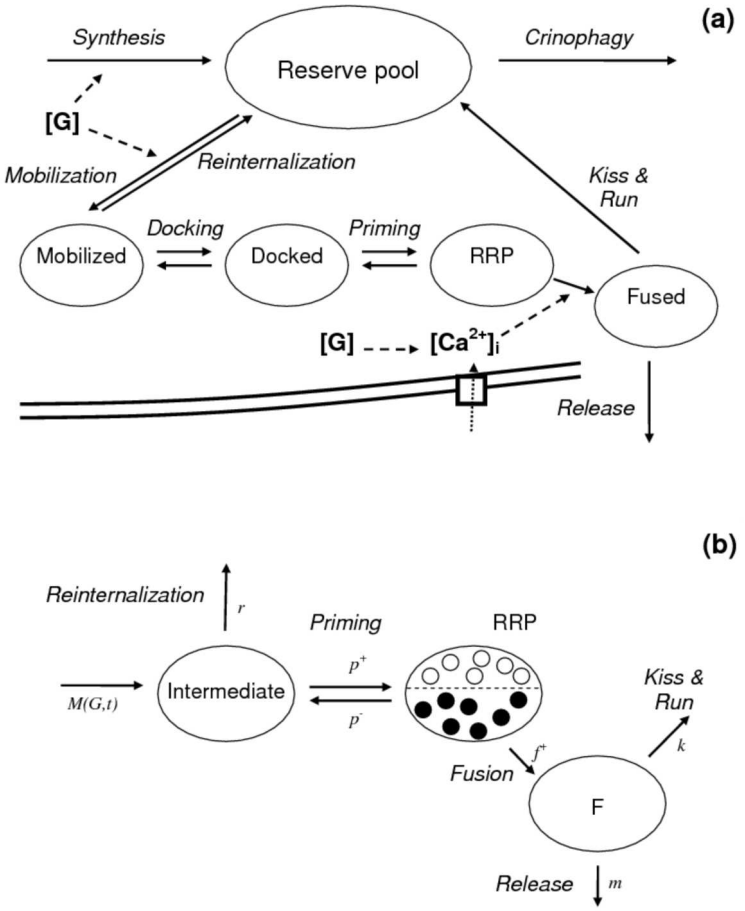

Figure 1. (a) Overview of granule pools and assumed glucose control points. Glucose ([G]) is assumed to control synthesis and mobilization. In addition, glucose promotes calcium influx through voltage gated calcium channels, which raises the intracellular calcium concentration ($[Ca^{2+}]_i$), the trigger of exocytosis. For further details, see the main text. (b) Schematic representation of the model [24]. The RRP has been divided into readily releasable granules located in silent cells with no Ca^{2+} influx, exocytosis and release (open circles) and readily releasable granules located in triggered cells (filled circles). Insulin is released from fused granules with rate constant independent of the glucose concentration and the threshold for activity of the cell.

for explaining data from in-vivo studies [30]. Derivative control arises from the threshold hypothesis as explained by Grodsky [15] and in greater detail by Licko [20]. We are currently investigating how derivative control arises in our mechanistic model [24], and how the subcellular parameters relate to those of the in-vivo model [30]. The aim is to be able to predict from in-vivo measurements, for example, which steps of glucose stimulated insulin secretion are impaired in diabetics. Such indirect knowledge of in-vivo β-cell functioning is virtually impossible without a mathematical model.

Two recent models go into greater details with respect to the different pools of granules in single cells [6, 11]. Both models include calcium, a necessity for coupling the granule model to models of bursting and calcium handling. Furthermore, the inclusion of calcium provides a way of modeling all steps from glycolysis via calcium dynamics to exocytosis and insulin release. Very recently, we extended the model by Chen *et al.* [11] by including a highly calcium-sensitive pool (HCSP) of granules [37, 38]. This allows us to connect recent imaging experiments with granule properties. It has been suggested that two different mechanisms operate during the two phases of biphasic insulin secretion, with transient first-phase secretion due to exocytosis of docked granules and the second sustained phase largely due to newcomer granules. We showed in [25] that the inclusion of an HCSP naturally leads to insulin secretion mainly from newcomer granules during the second phase of secretion, and found that the model is compatible with data from single cells on the HCSP and from stimulation of islets by glucose, including L- and R-type Ca^{2+} channel knock-outs, as well as from Syntaxin-1A deficient cells.

The mechanisms underlying various patterns of bursting electrical activity in response to different glucose stimuli are increasingly better understood with the use of mathematical models [5, 23]. The inclusion of a detailed description of insulin granules now allows modeling of events closer to secretion [6, 11, 24]. However, these models are based on data from rodents. It has recently been realized that human β-cells and islets differ substantially from rodent preparations [7,9]. Thus, to gain an understanding of the β-cell in diabetes these models should be updated as soon as more knowledge on human β-cells appears. Fridlyand *et al.* [14] studied the incretin effect on the oscillatory behavior. An important extension would be to include the effect of incretins on insulin secretion. One aim is to use the models to interpret in-vivo data more accurately as outlined above. Another aim is to construct insulin pumps that follow the dynamics of the natural pancreas more closely, for example, with respect to derivative control and pulsatile secretion. For such an "artificial pancreas", it would be important to include paracrine in-

teractions between α-, β- and δ-cells in the models [28]. Modeling of β-cells will likely move closer to clinical applications, where it can be expected to play an important role, as it continues to do in the understanding of the complex oscillatory phenomena observed in β-cells and islets.

3 Modeling Trafficking of Secretory Vesicles

Intracellular traffic and delivery of secretory granules to the plasma membrane play an important role in the process of secretion [25, 32]. Using the complex motion of vesicles, we estimate the vesicular exocytose rate, by taking into account the cytoskeletal organization of the cell.

The mathematical framework developed in [33] for modeling trafficking of membrane vesicles can naturally be extended to secretory granules. In this framework the cytosolic cellular compartment is represented as a domain Ω, which is approximated by a ball of radius R. Vesicles are secreted at the level of the Golgi apparatus and their movement can be seen as alternating between pure Brownian motion and deterministic movement along microtubules. For simplicity, we assume that microtubules emerge from the center of the cell and are radially organized in a symmetric way, ending at the cell surface. Although vesicles are of different sizes, we only consider vesicles of a mean radius a. Furthermore, there are plenty of vesicles in the cytoplasm (hundreds to thousands), but we neglect the fluctuation in their number and assume that as soon as a vesicle fuses, another is generated at the Golgi resulting in a constant supply of new vesicles. This assumption allows us to keep the number of vesicles constant.

We estimate the delivery rate, that is, the rate at which vesicles reach the cell surface, as follows. We evaluate the flux of vesicles to an exocytotic site $\partial\Omega_a$ and use the narrow escape theory described in [16]. This theory considers the mean time it takes a Brownian particle to arrive at a small absorbing surface $\partial\Omega_a$, while the particle is reflected at the remaining boundary. We approximate a vesicle as a round homogeneous sphere, so that its free movement is modeled here by the overdamped limit of the Langevin equation [26]. Furthermore, since a vesicle can also bind and then drift along microtubules with a deterministic velocity, its global motion is described by the stochastic rule

$$\dot{X} = \begin{cases} \sqrt{2D}\,\dot{w}, & \text{for } X(t) \text{ free}, \\ V(t)\,\mathbf{r}, & \text{for } X(t) \text{ bound}, \end{cases} \tag{1}$$

where $X(t)$ denotes the position and $V(t) \geq 0$ is a time-dependent drift velocity along microtubules directed toward the cell surface; we assume that

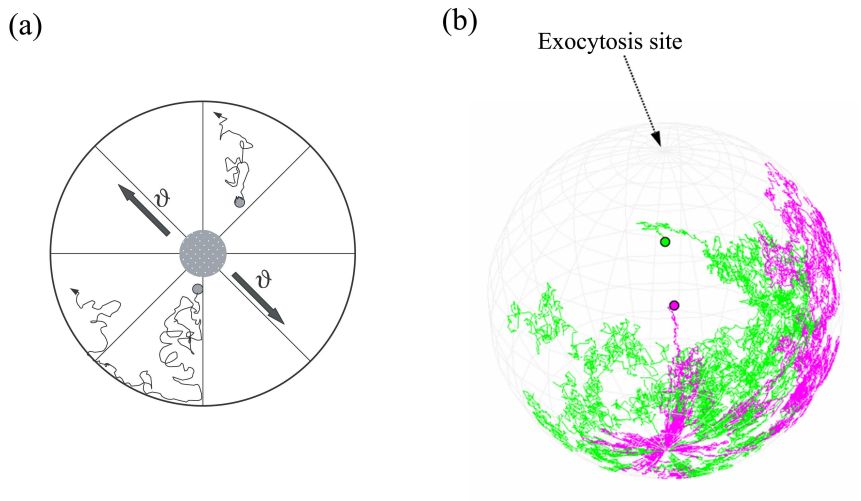

Figure 2. (a) Schematic representation of the vesicles' dynamics in the cell body. (b) Sample membrane vesicle trajectories in a three-dimensional cell produced by simulations of the homogenized version Eqn. (2) of the model.

the velocity along the microtubule is constant. The term w stands for δ-correlated standard white noise and $\mathbf{r}$ is the radial unit vector. In order to obtain an explicit expression for the vesicular flux to the site of exocytosis, we replace the vesicles' dynamics given by Eqn. (1) with a stochastic equation containing a steady-state drift $\phi(\mathbf{X})$,

$$\dot{\mathbf{X}} = \nabla\phi(\mathbf{X}) + \sqrt{2D}\,\dot{\boldsymbol{\omega}}. \tag{2}$$

Here, ϕ is the potential per unit mass, which generates the flow field velocity given by $\phi(\mathbf{X}) = \frac{1}{\gamma}(V\,\mathbf{r})$ and the diffusion constant is given by

$$D = \frac{k_B\,T_p}{m\,\gamma},$$

where m is the vesicle mass, γ the viscosity coefficient, T_p the temperature, and k_B is Boltzmann's constant. By using the distribution of vesicles at steady state, and the property that the surface of the exocytosis sites $\partial\Omega_a$ is small compared to the rest of the soma area, we compute the flux of vesicles to $\partial\Omega_a$. Then the vesicles' arrival rate, as derived in [33], is given by

$$\kappa_\delta = \frac{1}{\overline{\tau}_\delta} = \frac{\delta\,\vartheta}{\pi\left[R^2 - \frac{R\,D}{\vartheta} + \frac{D^2}{\vartheta}\right]}\,e^{-\Delta E/D},$$

where $\Delta E = \phi_m - \phi_0$. Note that, ϕ_0 and ϕ_m are both achieved on the cell surface, so $\Delta E = 0$. Moreover, the contribution of the last two terms in the denominator is negligible (it is around 1%). Hence, we get

$$\kappa_\delta = \frac{1}{\overline{\tau}_\delta} \approx \frac{\delta\,\vartheta}{\pi\,R^2}. \tag{3}$$

To derive this formula, the reader should keep in mind that the switching dynamics of the vesicle described by Eqn. (1) has been coarse grained by a stochastic equation with a radially constant drift given by Eqn. (2). We describe this procedure in detail in [33] and conclude that the effective drift ϑ captures the organization of the microtubules. Fig. 2 shows a schematic view of the model in panel (a) and sample trajectories of secretory vesicles in panel (b) produced by simulation of Eqn. (2).

4 Regulation of Calcium Concentration in Endocrine Cells

Exocytosis of secretory vesicles [2, 17, 36] depends on elevations in intracellular calcium concentration $[Ca^{2+}]_i$. In endocrine cells, the repetitive rises (oscillations) in $[Ca^{2+}]_i$ are accompanied by plateau-bursting electrical activity. Depending on the cell type, however, the bursting patterns can be rather distinct, leading to significant differences in the maximum levels of Ca^{2+} during a burst of action potentials. For example, the large-amplitude rapid spiking during the active phase of pancreatic β-cell bursts [1,3,17] can easily be distinguished from the small-spike amplitude in the active phase of pituitary cell bursters [34–36]. Furthermore, in contrast to pancreatic β-cells, whose rhythmic patterns have been extensively studied — see [5] for a review, — pituitary bursting is far less well understood. We discuss below recent insights in the regulation of plateau-bursting electrical activity in two types of pituitary cells, namely, somatotroph cells that secrete growth hormone and lactotroph cells that secrete prolactin.

4.1 Somatotroph Cells

In pituitary somatotrophs, modulation of the large-conductance calcium-sensitive potassium (BK) channels shows that decreasing the BK current, which is achieved in the mathematical model by increasing the parameter $b_{\rm BK}$, dramatically alters the duration of the active phase of the bursting electrical activity [34]. This voltage sensitivity consequently results in a decreased level of $[Ca^{2+}]_i$ that is ultimately associated with reduced hormonal

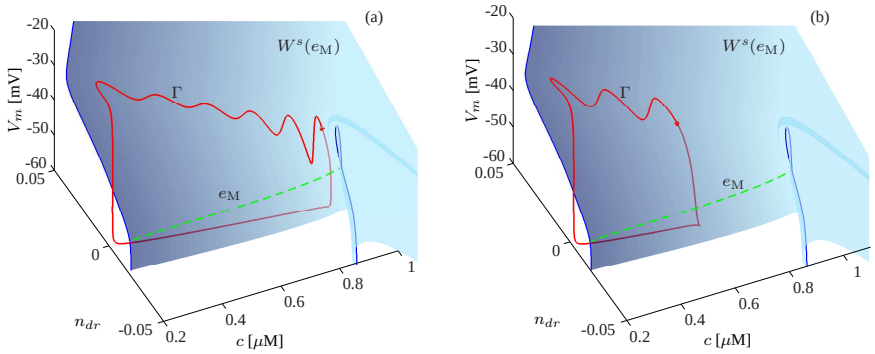

Figure 3. The c-dependent family $W^s(e_{\mathrm{M}})$ of one-dimensional stable manifolds of the saddle points along e_{M}. Panel (a) shows $W^s(e_{\mathrm{M}})$ for $b_{\mathrm{BK}} = 0$ and panel (b) for $b_{\mathrm{BK}} = 0.15$ along with the family e_{M} of saddle equilibria (green dashed line). The family $W^s(e_{\mathrm{M}})$ is shown as a blue gradient surface with two solid (blue) lines marking the bounding manifolds. The orbit Γ is depicted as a solid (red) curve. Reproduced from J. Nowacki, S. H. Mazlan, H. M. Osinga and K. T. Tsaneva-Atanasova, The role of large-conductance Calcium-activated K^+ (BK) channels in shaping bursting oscillations of a somatotroph cell model, *Physica D* (2009) © 2009, with permission from Elsevier.

secretion. The duration of the active phase depends on the mechanism that governs the termination of the plateau-bursting. Since blocking of BK channels does not significantly alter the underlying bifurcation diagram of the fast subsystem [34, Fig. 5], it cannot be the mechanism behind the termination of the active phase. However, for the range of c that corresponds to the active phase in [34, Fig. 5], we have coexisting (steady-state) attractors e_{H} and e_{L} in the fast subsystem that correspond to the active and passive phases, respectively. Therefore, the end of the active phase must be explained by the fact the trajectory Γ of the full system leaves the basin of attraction of e_{H}, thereby entering the basin of attraction of e_{L}.

The two-dimensional object in the full (V_m, n_{dr}, c)-space that separates the two basins of attraction is formed by the family of one-dimensional stable manifolds of points e_{M} on the middle branch of saddle equilibria. This family, denoted $W^s(e_{\mathrm{M}})$, is a well-defined manifold, because the branch of saddle points e_{M} and their associated one-dimensional manifolds depend smoothly on c. We compute $W^s(e_{\mathrm{M}})$ via continuation of a one-parameter family of two-point boundary value problems [19]. Figure 3 shows $W^s(e_{\mathrm{M}})$ as a blue gradient surface with the associated branch of equilibria e_{M} marked

by a dashed (green) line. Panel (a) shows the manifold for $b_{\mathrm{BK}} = 0$ and panel (b) for $b_{\mathrm{BK}} = 0.15$. In both cases the corresponding orbit Γ is shown as well (red curve).

Due to the presence of a homoclinic bifurcation for the fast subsystem, the corresponding saddle equilibrium must have the upper branch of its one-dimensional stable manifold fold over and connect back to this saddle equilibrium. Since the family of manifolds depends smoothly on c, one expects to see the folding happen earlier, for smaller values of c, so that the upper branch comes back below the corresponding saddle equilibrium and folds exponentially flat onto the lower branch. The start of this process can be observed in Fig. 3 as the darker shaded band running through the lighter side of $W^s(e_{\mathrm{M}})$, which is caused by the smaller steps taken in the numerical continuation to capture the dramatic change of the one-dimensional manifolds here. While the expected folding of the manifolds does take place before the homoclinic bifurcation, it happens only for a relatively small range of c-values and there is little difference between the manifolds for $b_{\mathrm{BK}} = 0$ and $b_{\mathrm{BK}} = 0.15$. This means that b_{BK} has no noticeable influence on the shape of the basin of attraction of active-phase equilibrium e_{H}. Hence, the parameter b_{BK} only influences the shape of the orbit Γ itself, such that its position with respect to $W^s(e_{\mathrm{M}})$ changes. Indeed, b_{BK} has the effect of increasing the amplitude of the oscilations of Γ during the active phase. This increase causes Γ to lie closer to $W^s(e_{\mathrm{M}})$ so that is crosses $W^s(e_{\mathrm{M}})$ for increasingly smaller values of c. As illustrated in Fig. 3, as soon as Γ crosses $W^s(e_{\mathrm{M}})$, it drops down to e_{L} and the active phase ends.

4.2 Lactotroph Cells

Lactotrophs are the anterior pituitary cells that secrete the hormone prolactin, which is involved in reproductive function and plays a role in immunity and metabolism. Like somatotrophs, lactotrophs are electrically active; they can generate spontaneous spikes or plateau bursts [36]. Calcium can enter the cell during plateau bursts and trigger hormone secretion. Thus, electrical activity plays an important role in basal hormone secretion. Modulators of prolactin secretion, such as estradiol and dopamine often target potassium (K^+) channels which affect the cell's excitability and, in turn, $[Ca^{2+}]_{\mathrm{i}}$ and prolactin secretion. Dopamine (in nanomolar concentration) inhibits lactotrophs, partly by opening several K^+ channels which decreases electrical activity [4]. However, low doses of dopamine (picomolar concentration) paradoxically stimulate prolactin secretion [8, 13]. How can activation of K^+ channels increase $[Ca^{2+}]_{\mathrm{i}}$ and stimulate prolactin secretion? Van Goor

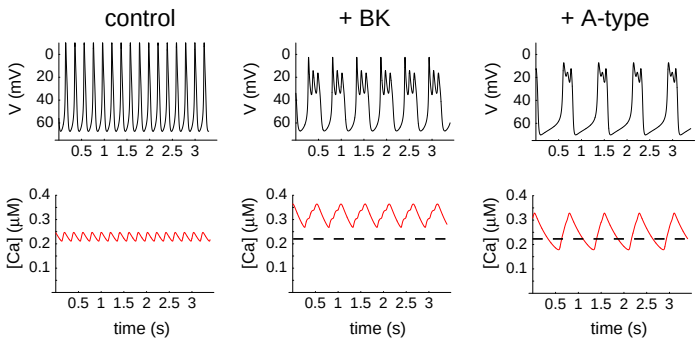

Figure 4. In the absence of BK or A-type channels, the lactotroph model is set to generate spontaneous spikes (left panels). Little Ca^{2+} enters the cell during a spike and average $[Ca^{2+}]_i$ is low. Addition of BK channels transforms the activity pattern to bursting (middle panels), with an increase in the average $[Ca^{2+}]_i$. Addition of the A-type channels also switches the activity pattern to bursting, but does not increase average $[Ca^{2+}]_i$. For details see [29].

et al. [35] showed that blocking the BK channels switches the pattern of somatotroph activity from bursting to spiking, correlated with a decrease in $[Ca^{2+}]_i$. If a low dose of dopamine activates mostly BK or equivalent K^+ channels, this could increase bursting and stimulate lactotrophs. For these reasons we investigate the role of the BK channels in the electrical activity of lactotrophs.

We built a model of a lactotroph cell based on a simple model for bursting in pancreatic β-cells in [27]. That is, we adjusted the parameters of the voltage-dependent Ca^{2+} and K^+ channels of the β-cell model to match parameters measured in lactotrophs and added basic Ca^{2+} dynamics. We also tuned the kinetics of the K^+ channels so that the model is spiking, not bursting. The addition of the BK channels, with fast voltage-dependent kinetics, transforms the electrical activity pattern from spiking to plateau bursting and increases $[Ca^{2+}]_i$; see Fig. 4. Adding more BK channels leads to longer bursts and higher $[Ca^{2+}]_i$ levels. Interestingly, this bursting activity has the same properties as the activity obtained from models of somatotroph cells [34, 35], such as low-amplitude spiking during the active phase. Unlike "classic" square wave bursting, where slow variations of $[Ca^{2+}]_i$ switch the system between a low steady state and a high, oscillatory (spiking) state, bursting in somatotrophs and lactotrophs involve transitions between two

steady states. The small-amplitude oscillations in the high state are due to a relatively slow rate of convergence to the high steady state because $[Ca^{2+}]_i$ dynamics are not as "slow" as in square wave bursting.

Another type of channel that is expressed by lactotrophs and can be modulated by dopamine is the A-type channel. It has fast voltage-dependent activation like BK channels, but also inactivates when membrane potential is maintained above rest. Adding A-type channels to the lactotroph model also switches the activity pattern from spiking to bursting. Also, $[Ca^{2+}]_i$ levels are slightly increased for a moderate addition of A-type channels. However, unlike for BK channels, adding more A-type channels leads to decreased $[Ca^{2+}]_i$ levels. Hence, BK and A-type channels act differently to produce bursting: BK activation speeds up the total K^+ current, leading to stabilization of high voltage levels, which necessitates a higher $[Ca^{2+}]_i$ level to terminate the bursts. On the other hand, the A-type current is mostly activated at lower voltage levels, which prevents spike/burst onset until $[Ca^{2+}]_i$ levels are sufficiently low [29]. Moreover, in the case of a moderate addition of A-type channels, bursting can continue even when $[Ca^{2+}]_i$ is clamped at a fixed value [31]. This is a new type of bursting that is not characterized by an obvious slow variable.

In summary, models of electrical activity in somatotrophs and lactotrophs are relatively new, but they have already revealed new bursting behaviors, for which some K^+ currents can have stimulatory effects.

5 Conclusions

Due to the fact that hormonal secretion is a very complex physiological process, modeling studies usually focus on a particular step or pathway involved in this phenomenon. However, the ultimate goal of understanding how the process works at a system's level requires the combination of these individual models and investigation of the complete system. A necessary first step toward achieving this goal is bringing together researchers modeling the mechanisms of secretion at different levels, from molecular to the level of the organ.

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